Academy of Romanian Scientists *Volume 14, No. 1, 2025, pp. 47-56*
Annals Series on Biological Sciences Online ISSN 2285 – 4177

ORIGINAL PAPERS

USING PASSIVE ACOUSTIC MONITORING TO ASSESS BIODIVERSITY PATTERNS IN A ROMANIAN BIOCULTURAL LANDSCAPE

Florina STĂNESCU[1,2,3], Teodora L. TĂNASE[1,3*]

[1] Ovidius University of Constanța, Faculty of Natural and Agricultural Sciences, Research Center of the Natural Sciences Department; Doctoral School of Applied Sciences, 1 Universității Al., 900470, Constanța, Romania
[2] Ovidius University of Constanța, Center for Research and Development of The Morphological and Genetic Studies of Malignant Pathology (CEDMOG), 900591 Constanţa, Romania
[3] Asociația Chelonia România, 5 Pascani Street, 062082 Bucharest, Romania
[*]Corresponding author e-mail: teodoral.tanase@365.univ-ovidius.ro

Abstract. *Passive acoustic monitoring (PAM) is a relatively modern approach with numerous applications in detecting biodiversity patterns and ecological research, in general. This study explored the diversity in a protected area from southeastern Romania, which shelters a unique blend of natural and cultural heritage. We conducted PAM over a one-month period and analyzed the acoustic data to assess the reliability of five most common acoustic indices used to characterize and monitor natural soundscapes. For this purpose, we also manually quantified the number of biophonies and vocalizing species, and the presence of noise (i.e., non-biological origin) in each recording. We found that only three of the acoustic indices were significantly correlated to the number of biophonies and vocalizing species. These indices reflect the level of disturbance (NDSI), diversity (AEI), and structure (H) in natural soundscapes. Noise, especially wind, affected the first 1 kHz frequency band, masking biophonies emitted within this range. Noise significantly influenced the acoustic indices, but the acoustic activity was also significantly lower during periods with strong wind, thunderstorms, or rain. We present the main challenges and solutions to overcome the limitations of PAM and provide a baseline description of the studied soundscape. We recommend integrating PAM in monitoring and management strategies of protected areas in Romania.*

Keywords: acoustic monitoring, biodiversity, indices, ecoacoustics, noise, soundscape.

DOI [10.56082/annalsarscibio.2025.1.47](https://doi.org/10.56082/annalsarscibio.2025.1.47)

Introduction

Wildlife monitoring has significantly evolved in the twenty-first century thanks to the technological advancement that enhances ecological research capabilities. Automated audio recorders stand out among these new technologies, revolutionizing traditional auditory-based survey methods [1]. Today, passive

acoustic monitoring (PAM) is a widely used non-invasive technique that uses automated sound recorders to monitor wildlife and entire ecosystems. The acoustic sensors can be deployed in the field for extended periods to record data on a preset schedule. Collected data is analyzed using dedicated softwares and can then be used to estimate a variety of biological and ecological metrics, from species richness, occupancy, abundance, population density and community composition to spatial and temporal trends in animal behavior [2, 1, 3].

Ecoacoustics is an expanding discipline that studies environmental sounds and can offer insights into the ecological patterns of acoustic communication ranging from individual organisms to whole ecosystems [4]. A foundational notion in ecoacoustics is the soundscape, which refers to the acoustic environment at a location, comprised of biological (biophony, e.g. vocalizing animals), geophysical (geophony, e.g. wind, rain) and anthropogenic sounds (anthropophony, sounds originating from human equipment/ activities, such as engine noise). Studying soundscapes provides insights into ecosystem functioning, integrity and complexity [5]. Biophonies are a major source of variation in natural soundscapes, making the use of acoustic indices potentially suitable to describe and track changes in ecological communities [6].

In Romania, biodiversity monitoring is still mostly based on classic methods, with modern approaches like acoustic monitoring still underutilized. As a consequence, there is a notable gap in published studies that use PAM, between Romania and other countries. The purpose of this study was to conduct an exploratory analysis of ecoacoustic diversity and evaluate the effectiveness of PAM as a tool for biodiversity monitoring within a protected area from southeastern Romania. Our objectives included (1) testing the validity of some of the most frequently used acoustic indices in ecoacoustics and (2) identifying potential limitations and challenges of PAM.

Materials and methods

Study area and sampling. The Histria Archaeological Complex (HAC), located on the western coast of the Black Sea in Romania, is mostly known for its historical and cultural significance. Established in the 7th century BC by Greek colonists, Histria is one of the oldest urban settlements in Romania. Over centuries, it has served as a key center for various civilizations, including the Greeks, Romans, and Byzantines, each contributing to its rich cultural and architectural heritage. The complex covers a 32-ha area of sandy inland dunes covered by steppe vegetation and surrounded by wetlands [7]. It is located in the southern part of the Danube Delta Biosphere Reserve (DDBR), one of Europe's most extensive and well-preserved deltas. Despite its documented archaeological importance [8], the natural heritage at this site has received comparatively less

attention [9, 10, 11]. This area, although managed independently, is located within "Istria-Sinoe Lagoon System", one of the 20 strictly protected areas of DDBR. The lagoon system covers 400 ha of wetland crossed by sand levees, which supports high biodiversity [10], including several species listed under Annex II of the Habitats Directive within the Natura 2000 Network.

We used an automated sound recorder, Audiomoth v.1 [12], to record three minutes every hour, daily, from March 29 to April 24 2023 (sampling location: 44.549274, 28.765082). We set the sample rate at 96 kHz and the microphone gain on the „medium" level. The recordings obtained in this study are deposited in the sound collection of the Department of Natural Sciences, Ovidius University of Constanta, and can be accessed by request from the senior author.

Data analysis. We used Raven Pro 1.6 [13] to visualize spectrograms (window size = 512 points) and listen to audio files. First, we manually extracted the following biological information for each three-minute recording: the presence of noise (i.e., as a proxy for disturbance), number of biophonies (i.e., as a proxy for abundance), and number of vocalizing species and higher taxa (i.e., as a proxy for diversity). Anthropogenic and geophysical sounds were classified as "noise" (i.e., aircraft, motor vehicles, human voices, wind, rain, thunder), while animal sounds were counted and categorized by taxa (i.e., Amphibians, Birds, Insects, Mammals). We grouped the audio samples into three time periods: 3-10 AM (dawn), 11 AM - 6 PM (midday) and 7 PM - 2 AM (dusk), to assess and describe the diurnal patterns in the soundscape.

We used the statistical software R [14], with the function "multiple_sounds" from the package "soundecology" [15] to compute the following five acoustic indices, most commonly used to characterize and measure soundscape diversity, complexity and perturbance: the Acoustic Complexity Index (ACI), the Bioacoustic Index (BI), the Acoustic Evenness Index (AEI), the Total Entropy Index (H, from the package "seewave"; [16]) and the Normalized Difference Soundscape Index (NDSI) [17, 18]. Acoustic indices are quantitative measures used to characterize soundscapes, providing insights into biodiversity patterns and the presence/ impact of anthropogenic disturbance. Statistical tests were performed using the IBM SPSS Statistics software [19].

Following the visual analysis of the spectrograms in Raven Pro, we selected the first 1 kHz frequency band as representative of anthropogenic sounds and geophonies, with most of the biophonies occurring above this threshold. Thus, all acoustic indices were computed at a window size of 512 points, with biophonies set between 1.1 and 48 kHz. In the case of the Total Entropy Index, there was no option to define the frequency bands for noise and biophonies. As such, this index was computed for the entire frequency spectrum.

The Acoustic Complexity Index (ACI) [20], captures the variability in sound intensity typically seen in biotic sounds, like bird songs, while distinguishing these from human-made noises that tend to have more consistent intensity levels. We computed this index with the cluster size set at 30 s. The Acoustic Evenness Index (AEI) [21] computes the evenness of sound energy distribution across frequency bins, to express the relative balance among sound sources in the acoustic environment. Its values range from 0 to 1, where values closer to 0 indicate higher evenness (e.g., in acoustically saturated environments, with little variation in intensity among frequency bands) [6]. For this index, we set the size of frequency bands at 1 kHz. The Bioacoustic Index [22] quantifies the acoustic energy of a soundscape by calculating the area under each curve for each frequency band. This reflects the sound level and frequency diversity linked with biophonies. The Total Entropy Index (H) returns a single value within the range [0, 1], where the entropy of a less structured acoustic environment will approach 1 (e.g., noisy or acoustically saturated environments). The Normalized Difference Soundscape Index (NDSI) compares the ratio of noise (i.e., anthropogenic sounds or geophonies; defined by the user) to biological sounds in a sample, providing a measure of disturbance; the results range between -1 and +1, where -1 indicates a disturbed soundscape [23].

Results

During the one-month monitoring period, we recorded a total of 31.1 hours of audio data (i.e., 622 three-minutes recordings). The dataset encompassed a wide range of vocalizations, allowing for a comprehensive analysis of the site's acoustic diversity. The number of biophonies and the number of vocalizing species were significantly higher during dawn and midday, compared to dusk (Kruskal-Wallis test, number of biophonies: χ^2=93.011, df=2, p<0.001, Fig. 1; number of species: χ^2=91.711, df=2, p<0.001; Fig. 2).

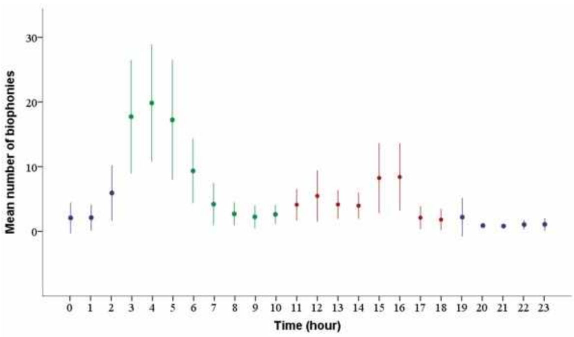

Fig. 1. The diurnal variation in the mean number of biophonies during the sampling period (29 March to 24 April 2023), at Histria Archaeological Complex. The three time periods are represented in: blue (dusk), green (dawn) and red (midday). The whiskers represent the 95% confidence intervals.

Birds were the most active acoustic group, present in more than half (53%) of the recordings, followed by amphibians (19%), mammals (11%) and insects (7%). We recorded the presence of domestic dogs in 54 out of the 622 recordings (i.e., 9%). The diurnal pattern of acoustic activity varied across taxonomic groups (Fig. 3). Bird vocalizations were predominant between 2 AM and 6 PM, with peak activity observed around 4 AM. Amphibian calls were most frequent during the evening and early night hours (4 PM to 2 AM). Mammals (i.e., dogs, jackals, bats) were more active between 10 PM and 1 AM. Insect sounds were primarily recorded during daylight hours, between 7 AM and 4 PM. Noise (i.e., engine noises, strong wind, thunderstorm, rain) was present in 213 of the 622 recordings (i.e., 34%), mostly during dawn and midday.

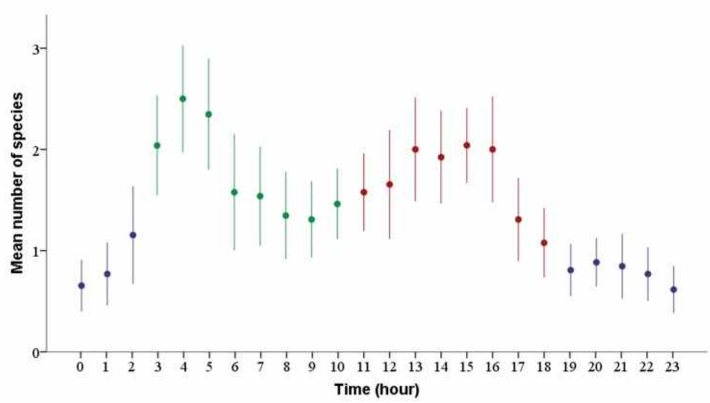

Fig. 2. The diurnal variation in the mean number of vocalizing species during the sampling period (29 March to 24 April 2023), at Histria Archaeological Complex. The three time periods are represented in: blue (dusk), green (dawn) and red (midday). The whiskers represent the 95% confidence intervals.

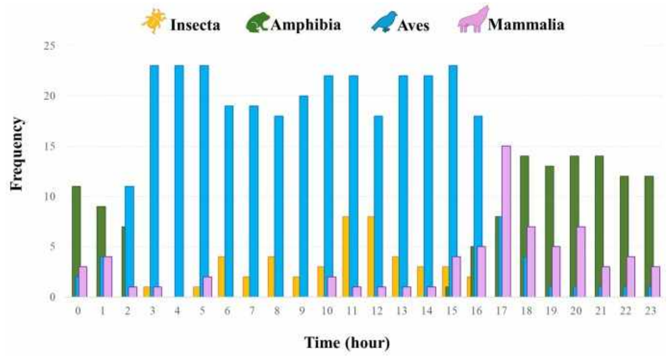

Fig. 3. The diel variation in the frequency of vocalizing taxa (presence records based on the acoustic activity) during the sampling period (29 March to 24 April 2023), at Histria Archaeological Complex. The taxonomic groups are represented in: orange - Insecta, green – Amphibia, blue – Aves, and pink - Mammalia.

We found significant correlations between three (i.e., NDSI, H and AEI) of the five acoustic indices and the biological parameters (i.e., number of biophonies and species), although the correlation coefficients were low. These three indices were also highly correlated with each other (Table 1). The correlation with the biological parameters was positive in NDSI and H; thus, a less disturbed and less structured soundscape is richer in biophonies and vocalizing species. The AEI was negatively correlated with the number of biophonies; thus, a higher evenness in the distribution of sound across frequencies (i.e., lower AEI values) corresponds to a soundscape richer in biophonies. All acoustic indices were significantly affected by noise (Table 1); however, the presence of noise also significantly reduced the number of biophonies (Spearman's *rho* = -0.225, $p < 0.001$) and vocalizing species (Spearman's *rho* = -0.220, $p < 0.001$).

Table 1. Correlation tests results, showing Spearman's *rho* and the level of significance. NDSI = the Normalized Difference Soundscape Index; ACI = the Acoustic Complexity Index; BI = the Bioacoustic Index; H = the Total Entropy Index; AEI = the Acoustic Evenness Index; Bio = number of biophonies; S = number of species; ** = correlation is significant at the 0.01 level (2-tailed); * = correlation is significant at the 0.05 level (2-tailed). Significant correlations are marked in bold for the relevant indices.

	NDSI	ACI	BI	H	AEI	Bio	S	Noise
NDSI	1	-0.628**	-0.510**	0.958**	-0.898**	**0.096***	**0.089***	**-0.342****
ACI	-0.628**	1	0.813**	-0.748**	0.538**	0.008	0.024	0.329**
BI	-0.510**	0.813**	1	-0.655**	0.417**	0.036	0.044	0.347**
H	0.958**	-0.748**	-0.655**	1	-0.862**	**0.095***	**0.094***	**-0.360****
AEI	-0.898**	0.538**	0.417**	-0.862**	1	**-0.096***	-0.075	**0.231****

The acoustic indices revealed distinct patterns of acoustic activity that varied across time periods. The NDSI revealed a significantly less perturbed soundscape during dusk, compared to both dawn and midday (Kruskal-Wallis test, χ^2=28.036, df=2, p<0.001, see Figure 4); no significant differences were found between dawn and midday.

The AEI was significantly lower during dusk, indicating higher evenness within the soundscape, compared to both dawn and midday (Kruskal-Wallis test, χ^2=22.435, df=2, p<0.001, see Figure 5); no significant differences were found between dawn and midday. This pattern can be also observed in the frequency of vocalizing taxa (see Figure 3), where the dusk soundscape is characterized by acoustic signals scattered across multiple frequency bands – the lower frequencies are mostly occupied by amphibians and part of the mammals (e.g., dogs, jackals),

the mid-frequency band is used by nocturnal bird species, while the mid and highest frequency bands are occupied by bats.

In terms of entropy (H), we found significant differences between all three time periods of the day (Kruskal-Wallis test, χ^2=26.650, df=2, p<0.001). The dusk soundscape was characterized by the highest entropy, indicating a more saturated acoustic activity, followed by midday and dawn; the dawn soundscape had the lowest entropy, indicating a more structured soundscape (i.e., biophonies concentrated in fewer frequency bands) (see Figure 6). This is in accordance to the acoustic activity of birds, which dominate the dawn and midday soundscape; thus, the acoustic energy occupies a narrower frequency range.

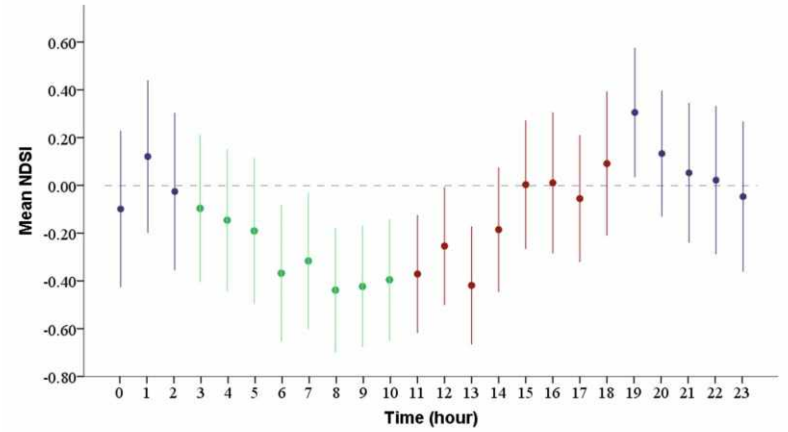

Fig. 4. The diurnal variation in NDSI (average values) during the sampling period (29 March to 24 April 2023), at Histria Archaeological Complex. The three time periods are represented in: blue (dusk), green (dawn) and red (midday). The whiskers represent the 95% confidence intervals.

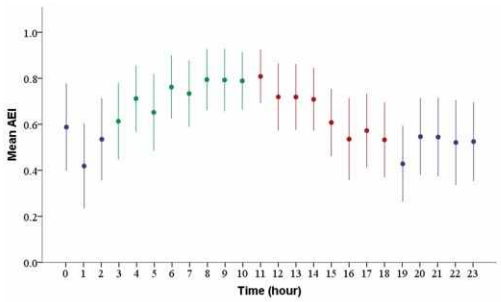

Fig. 5. The diurnal variation in AEI (average values) during the sampling period (29 March to 24 April 2023), at Histria Archaeological Complex. The three time periods are represented in: blue (dusk), green (dawn) and red (midday). The whiskers represent the 95% confidence intervals.

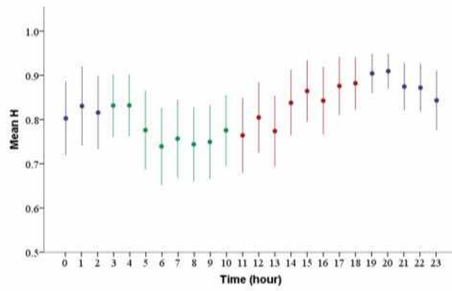

Fig. 6. The diurnal variation in H (average values) during the sampling period (29 March to 24 April 2023), at Histria Archaeological Complex. The three time periods are represented in: blue (dusk), green (dawn) and red (midday). The whiskers represent the 95% confidence intervals.

Discussion

We conducted a PAM study in Romania aimed at assessing the reliability of acoustic indices for ecological monitoring in a protected area from the Danube Delta Biosphere Reserve. We validated three of the most frequently used acoustic indices in ecoacoustics, which reflect the level of disturbance (NDSI), variation/ diversity (AEI), and structure (H) in natural soundscapes. These indices showed significant correlations with the abundance and diversity of vocalizing species, supporting their reliability as biodiversity indicators [1]. Hence, we demonstrate a high potential of integrating PAM in the monitoring and management strategies of protected areas in Romania. This is an accessible and practical approach for protected areas administrators, requiring basic knowledge of bioacoustics and statistics.

One of the main challenges we identified was related to noise. At Histria Archaeological Complex, noise was predominant in the first 1 kHz frequency band. Anthropophonies and geophonies, especially strong wind, masked the bioacoustic signals emitted at low frequencies (e.g., by amphibians, some birds and jackals). On the other hand, we found that animal communication was also significantly lower during strong wind, thunderstorms or rain. For long-term monitoring and comparison purposes, PAM should be conducted during similar periods of time (e.g., time of the year/ day), using similar settings (e.g., sampling rate, microphone gain). The data selected for analyses should be from recordings obtained under similar conditions, ideally without strong wind, rain, or other strong, non-biological acoustic signals. The use of windscreens (i.e., for microphones, or the entire protective case) can improve the quality of the recordings. Other challenges are related to the high volume of data and its management. We highly recommend depositing the recordings and associated metadata in open-access libraries or/ and creating and maintaining an organized soundscape library.

Our study provides baseline data and information regarding the soundscape characteristic to a protected area of biocultural importance. To the best of our knowledge, this is the first published study for Romania, where datasets obtained through PAM are used to assess biodiversity and disturbance patterns at soundscape level. We highlighted the peaks in the acoustic activity of four animal groups and showed that birds and amphibians were the most active groups during the sampling period. Birds were most vocal during dawn and midday, while amphibians were the predominant group during dusk. Although peak levels of noise disturbance overlapped with the peak acoustic activity of birds, bird vocalizations were mostly concentrated above the noise threshold. However, this is not valid in amphibians and mammals that occupy the lower frequency bands of the acoustic space. For example, most anurans evolved to have the best auditory sensitivity and peak spectral energy in the lower frequency bands (< 2 kHz) of the acoustic space [24]. Previous research showed that anthropogenic noise overlapping the acoustic space of amphibians interfered with their acoustic communication and the acoustic properties of the advertisement calls [e.g., 25]. Acoustic communication is of paramount importance for succesful reproduction and survival in most anurans. Thus, wildlife managers should account for noise pollution as a significant negative factor during the breeding season of sensitive species.

Conclusions

This study demonstrates the applicability of passive acoustic monitoring as a valuable tool for understanding and managing the biodiversity of protected areas. Current challenges are related to environmental noise interference and "big data" management. Future research should focus on improving PAM technologies and analytical tools, and expanding its use across different ecosystems. Such advancements will allow us to gain deeper insights into biodiversity patterns and its response to environmental changes, while also guiding more effective, targeted conservation strategies.

Acknowledgments. This study was supported by The Academy of Romanian Scientists through an AOSR-TEAMS grant (2023-2024; FS) and Asociația Chelonia Romania. We highly appreciate the valuable insights provided by prof. Dan Cogălniceanu.

R E F E R E N C E S

[1] L.S.M. Sugai et al., *BioScience,* 69(1), 15-25 (2019).

[2] E. Browning et al*., WWF Conservation Technology Series,* 1(2), 1 (2017).

[3] W. Penar et al., *Ecological Complexity,* 43, 100847, (2020).

[4] T. Bradfer-Lawrence et al., *Methods in Ecology and Evolution,* 14, 2192-2204, (2023).

[5] T. Bradfer-Lawrence et al., *Methods in Ecology and Evolution*, (2024). https://doi.org/10.1111/2041-210X.14357

[6] I. Alcocer et al., *Biological Reviews,* 97(6), 2209-2236, (2022).

[7] S. E. Vlad et al., *Amphibia-Reptilia,* 45(3), 265-277, (2024).

[8] A. Vespremeanu-Stroe et al., *Quaternary International,* 293, 245-256, (2013).

[9] D. Cogălniceanu et al*., ZooKeys,* (341), 49, (2013).

[10] R. Iosif et al., *Human Dimensions of Wildlife,* 24 (4), 301-313, (2019).

[11] S. E. Vlad et al., *Herpetology Notes*, 13, 523-525, (2020).

[12] A. P. Hill et al., *Methods in Ecology and Evolution,* 9(5), 1119-12111, (2018).

[13] K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, Raven Pro: Interactive Sound Analysis Software (Version 1.6.5) [Computer software] (Cornell Lab of Ornithology, Ithaca, NY, 2024). https://www.ravensoundsoftware.com/.

[14] R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

[15] L. J. Villanueva-Rivera and B. C. Pijanowski*,* 1(3), 3 (2018). https://CRAN.R-project.org/package=soundecology.

[16] J. Sueur et al., *Bioacoustics*, 18, 213 (2008).

[17] T. Bradfer-Lawrence et al., *Ecological Indicators,* 115, 106400, (2020).

[18] B. Pu et al., CELA 2023 San Antonio: Align | Realign, (2024).

[19] IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

[20] N. Pieretti et al., *Ecological Indicators,* 11(3), 868-873, (2011).

[21] L. J. Villanueva-Rivera et al., *Landscape Ecology,* 26, 1233-1246, (2011).

[22] N. T. Boelman et al., *Ecological Applications,* 17(8), 2137-2144, (2007).

[23] E. P. Kasten et al., *Ecological Information,* 12, 50-67, (2012).

[24] A.M. Simmons and P.M. Narins, *Springer Handbook of Auditory Research*, 66, (2018). https://doi.org/10.1007/978-1-4939-8574-6_7

[25] V. Zaffaroni-Caorsi et al., *Bioacoustics*, 32(1), 90-120, (2023).